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16. ABSTRACT

Daphnia magna, the crayfish Pacifastacus leniusculus, and nymphs of the stoneflies, Acroneuria californica, A. pacifica, and Pteronarcys californica were tested in the laboratory to determine their survival in different concentrations of air-supersaturated water. The mean 96-h LC50 value (lethal concentration, 50% mortality) for D. magna was 122.5% total air saturation. The 7-day LC50 was 120% and the 10-day LC50 was 117.5%. The mean 96-h LC50 for crayfish was 147% total air saturation. The 7-day LC50 was 145% and the 10-day LC50 was 133%. The 10-day EC50 values (effective concentration, 50% immobilized) for stoneflies were 135% for A. californica and greater than 125% for A. pacifica and P. californica. Bubbles (emboli) were observed in body fluids and tissues, and general body distention occurred before death in Daphnia, crayfish, and stoneflies. Lethal threshold concentrations for Daphnia and crayfish were near 111 and 127%, respectively.

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Survival of *Daphnia*, Crayfish, and Stoneflies in Air-Supersaturated Water

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Daphnia magna, the crayfish *Pacifastacus leniusculus*, and nymphs of the stoneflies, *Acroneuria californica*, *A. pacifica*, and *Pteronarcys californica* were tested in the laboratory to determine their survival in different concentrations of air-supersaturated water. The mean 96-h LC50 value (lethal concentration, 50% mortality) for *D. magna* was 122.5% total air saturation. The 7-day LC50 was 120% and the 10-day LC50 was 117.5%. The mean 96-h LC50 for crayfish was 147% total air saturation. The 7-day LC50 was 145% and the 10-day LC50 was 133%. The 10-day EC50 values (effective concentration, 50% immobilized) for stoneflies were 135% for *A. californica* and greater than 125% for *A. pacifica* and *P. californica*. Bubbles (emboli) were observed in body fluids and tissues, and general body distention occurred before death in *Daphnia*, crayfish, and stoneflies. Lethal threshold concentrations for *Daphnia* and crayfish were near 111 and 127%, respectively.

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Nous avons fait des expériences en laboratoire avec *Daphnia magna*, l'écrevisse *Pacifastacus leniusculus* et les nymphes de perles *Acroneuria californica*, *A. pacifica* et *Pteronarcys californica*, dans le but de déterminer leur survie dans de l'eau sursaturée d'air. La CL50 moyenne après 96 h (concentration létale, 50% de mortalités) pour *D. magna* est de 122.5% de la pleine saturation d'air. La CL50 après 7 jours est de 120%, et la CL50 après 10 jours est de 117.5%. La CL50 moyenne après 96 h pour l'écrevisse est de 147% de la pleine saturation d'air. La CL50 après 7 jours est de 145% et la CL50 après 10 jours est de 133%. Les CE50 après 10 jours (concentration effective, 50% immobilisés) pour les perles sont de 135% pour *A. californica* et supérieures à 125% pour *A. pacifica* et *P. californica*. Nous avons observé des bulles (embolies) dans les humeurs et les tissus, et il se produit un gonflement général du corps avant la mort chez les daphnies, les écrevisses et les perles. Les concentrations de seuil létales pour les daphnies et les écrevisses sont près de 111 et 127% respectivement.

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THE effects of air-supersaturated water on aquatic organisms, the so-called gas-bubble disease (GBD), have been familiar to aquarium and hatchery workers for many years (Marsh and Gorham 1905; Embody 1934; Rucker and Hodgeboom 1953). Supersaturated water (up to 150% total dissolved gas) created by dams on the Columbia River, and its effect on fish, have recently been described by Westgard (1964), Pauley and Nakatani (1967), Ebel (1969), and others. Three recent literature reviews (Rucker 1972; Weitkamp and Katz 1973; Bouck 1973) adequately summarize previous work on GBD and supersaturated water problems. Laboratory studies have been conducted with fish (Nebeker and Brett 1976) but little information is available for fish food organisms, especially freshwater invertebrates. Gorham (1901) reported that scallops, hydroids, and squids showed signs of GBD. Evans and Walder (1969) used the shrimp *Crangon* to study bubble formation under decompression. Hughes (1968) reported GBD in lobsters exposed to water supersaturated by air leaking into a hatchery water supply, and Malouf et al. (1972) described the occurrence of GBD in three species of bivalve molluscs. Gas-bubble disease in larval and juvenile brown shrimp was described by Lightner et al. (1974). No saturation levels were given with any of the above observations. Time to death at several measured gas concentrations has been given for several invertebrate species by Nebeker et al. (1975).

This study was conducted to determine the effects of several measured levels of air supersaturation on the survival of the following freshwater fish food organisms: juvenile and adult *Daphnia magna*; juvenile and adult western crayfish *Pacifastacus leniusculus*; and nymphs of the stoneflies, *Acroneuria californica*, *A. pacifica*, and *Pteronarcys californica*.

TABLE 1. Nominal and measured test levels of total gas supersaturation.

Test no.	Nominal total percent saturation[b]	Measured total percent saturation[a]		
		N	$\bar{x}$	$\pm$ SD
1	140	5	140.6	1.4
	130	5	130.5	1.1
	120	5	119.4	0.5
2	150	5	150.2	0.9
	140	7	139.8	1.1
	130	10	128.6	1.4
	120	10	119.1	0.8
3	120	3	119.2	1.1
	115	3	115.4	0.6
	115	3	114.8	0.4
	110	3	108.7	0.6
4	125	45	124.7	0.7
5	150	5	148.8	1.1
6	150	3	150.4	1.2
	140	21	141.4	1.5
	130	25	130.2	1.2
	120	29	119.4	0.8
7	150	11	150.9	1.1
	140	10	139.9	0.9
	120	12	119.3	0.8
8	125	45	124.7	0.7
9	135	4	135.5	0.4

[a]Analysis with Weiss saturometer.
[b]Control tanks remained near 100% saturation.

Materials and methods — A supersaturation generator system was used to produce and control the air-supersaturation levels in each test tank (Nebeker et al. 1975). Test water was aerated to saturation, heated to required test temperature, pumped under pressure, and mixed with compressed air. Water and any remaining air not in solution flowed through the

FIG. 1. *Daphnia* exhibiting signs of gas-bubble disease. Upper and lower animals have air bubbles in the gut; the center animal is the control.

retention tank where excess air was vented off. Test water then flowed into the exposure tank where it became supersaturated when released back to normal atmospheric pressure. The degree of supersaturation was controlled by the amount of compressed air added to the water. Test animals were held in cages or allowed to move freely within the test tanks.

A Weiss saturometer was used for measuring total dissolved air saturation concentrations in the test tanks (Table 1). It was calibrated with a Van Slyke gas analyzer and Winkler dissolved oxygen determinations. The formula $\frac{BP + \Delta P - VP}{BP} \times 100 = \%$ saturation, where BP = barometric pressure, ΔP = saturometer reading, and VP = water vapor pressure, was used to calculate total percent saturation.

Test water was relatively soft with the following mean chemical characteristics: hardness (as $CaCO_3$) 34 mg/liter; alkalinity (as $CaCO_3$) 31 mg/liter; pH, 6.7. The pH increased to 7.2–7.4 after water aeration. Complete analyses of test water were summarized by Samuelson (1976). Deaths (criteria used were lack of a heartbeat or lack of movement when disturbed) and signs of GBD were recorded daily and data were analyzed according to APHA et al (1971).

Prior to experimentation *Daphnia magna* were obtained from cultures maintained at the Western Fish Toxicology Station and held at 15 ± 2 C. Water was changed biweekly. Crayfish and stoneflies were collected from local streams and acclimated in the laboratory at 11–12 C for at least 24 h. Both species were held in tanks with flowing water. Supersaturation concentrations were set at the desired levels and test animals were placed directly into the test tanks. Three tests with *Daphnia* were completed at concentrations of 150, 140, 130, 120, 115, 110, and 100% total air saturation. Four tests with crayfish were

TABLE 2. Survival of *Daphnia*, crayfish, and stoneflies in air-supersaturated water at 12 C.

Species	Test no.	Test conditions	Exposure time	LC50[a]	Concn (total percent air saturation)
Daphnia magna	1	—[b]	96 h	LC50	114.0%
	2	—[c]	96 h	LC50	135%
		—[c]	7 day (168 h)	LC50	120%
		—[c]	10 day (240 h)	LC50	117.5%
	3	—[c]	96 h	LC50	118.5%
Crayfish *Pacifastacus leniusculus*	6	—[b]	96 h	LC50	143%
	7	—[b]	96 h	LC50	151%
			7 day (168 h)	LC50	145%
			10 day (240 h)	LC50	133%
Stoneflies *Acroneuria californica*	9	—[b]	10 day (240 h)	EC50	135%
Acroneuria pacifica	8	—[b]	10 day (240 h)	EC50	>125%
Pteronarcys californica	8	—[b]	10 day (240 h)	EC50	>125%

[a]LC50 = lethal concentration (50% mortality); EC50 = effective concentration (50% immobilized). (Calculated as outlined by the APHA et al. 1971).

[b]In flowing water with no food.

[c]In still water with food.

TABLE 3. Mean time to 50% and 20% death for *Daphnia*, crayfish, and stoneflies. (* Not achieved — insufficient number of deaths.)

Test animal and test no.	Nominal percentage saturation	Mean time to 50% death (*h*)	Mean time to 20% death (*h*)
Daphnia			
1	140	71	48
	130	65	43
	120	91	38
2	150	101	82
	140	123	72
	130	130	92
	120	210	131
3	120	93	49
	115	*	137
	110	*	*
Crayfish			
4	125	*	*
5	150	—	35
6	150	94	40
	140	330	130
	130	*	454
	120	*	*
7	150	123	66
	140	165	122
	120	*	*
Stoneflies			
8	125	*	*
9	135	*	*
	120	*	*
	115	*	*

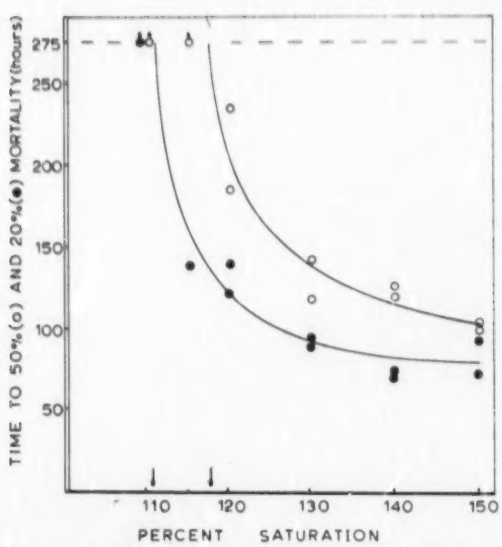

FIG. 2. Lethal threshold concentrations determined for *Daphnia magna* using time to 50 and 20% mortality to plot the curves.

conducted at test levels of 150, 140, 130, 125, 120, and 100% saturation. Two tests at 135, 125, 120, 115, and 100% saturation were conducted with the stoneflies. All tests were at 12 C under natural photoperiod. Additional details of individual experiments are given in Table 2.

Results and discussion — Daphnia tests — Daphnia magna were affected by supersaturated water above 110% total air saturation, and signs of GBD ranged from no obvious visual effects to massive air bubbles in the gut, brood pouch, and under the carapace (Fig. 1). The mean 96-h LC50 value for *D. magna* was 122.5% total air saturation when *Daphnia* were fed and held in quiet water. When they were held in flowing water and were not fed the 96-h LC50 was 114.0%. In one test the 7-day LC50 value was 120% and the 10-day LC50 was 117.5% (Table 2). The most obvious sign of GBD was buoyancy created by bubbles that caused Daphnia to float to the water surface. The heart was apparently unaffected and bubbles were rarely observed in the body fluid. Lethal threshold concentrations for *Daphnia*, calculated from time to 50% death in supersaturated water (Table 3) were deter-

mined (Fig. 2). The level at which 20% of the *Daphnia* were killed, near a possible safe level, was near 111% total dissolved gas.

Crayfish tests — Crayfish were affected by supersaturated water above 130% total air saturation, and signs of GBD, as in *Daphnia*, ranged from no obvious visual effects to massive blockage of body fluids and air bubbles (emboli) in gills and other body structures. Emboli did occur in the heart of the crayfish at 150% causing the death of the animals. They were immobilized for many hours before death as they became turgid and swollen from internal pressures of gas and from water taken up due to osmotic imbalance.

The mean 96-h LC50 determined for crayfish was 147% total air saturation. The 7-day LC50 and 10-day LC50 were 145 and 133%, respectively, obtained in a different test. A lethal threshold concentration for crayfish, using time to 20% death, was near 127% total dissolved gas.

Stonefly tests — The 10-day EC50 values for stoneflies were 135% for *A. californica* and greater than 125% for *A. pacifica* and *P. californica*. Stoneflies held at 115% exhibited no signs of stress; those held at 125% had problems with external bubbles buoying them to the water surface. Half the stoneflies held at 135% had bubbles in the body fluids, and their bodies were distended to the capacity of the body wall. Bubbles were visible through the body wall at the base of the legs, the gills, and other areas such as the mandibles. Increased buoyancy was observed frequently and may increase deaths of insects due to predation.

The open circulatory system of invertebrates, relatively simple compared to fish, may be a possible reason for the greater tolerance of stoneflies and crayfish to GBD. Also, the tough exoskeleton of insects and crustacea prevents much of the surface injury and secondary infections that are common to fish exposed to supersaturated water.

AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. AMERICAN PUBLIC HEALTH ASSOCIATION, 1971. Standard methods for the examination of water and wastewater. 13th ed. New York, N.Y. 874 p.

BOUCK, G. R. 1973. Total dissolved gas pressure, p. 135–139. *In* Water quality criteria. National Academy of Sciences. EPA-R3-73-033.

EBEL, W. J. 1969. Supersaturation of nitrogen in the Columbia River and its effect on salmon and steelhead trout. Fish. Bull. 68: 1–11.

EMBODY, G. C. 1934. Relation of temperature to the incubation period of eggs of four species of trout. Trans. Am. Fish. Soc. 64: 281–292.

EVANS, A., AND D. N. WALDER. 1969. Significance of gas micronuclei in the aetiology of decompression sickness. Nature 222: 251–252.

GORHAM, F. P. 1901. The gas bubble disease of fish and its cause. Bull. U.S. Fish. Comm. (1899). 19: 33–37.

HUGHES, J. T. 1968. Grow your own lobsters commercially. Ocean Ind. 3: 46–49.

LIGHTNER, D. V., B. R. SALSER, AND R. S. WHEELER. 1974. Gas-bubble disease in the brown shrimp (*Penaeus aztecus*). Aquaculture 4: 81–84.

MALOUF, R., R. KECK, D. MAURER, AND C. EPIFANIO. 1972. Occurrence of gas-bubble disease in three species of bivalve molluscs. J. Fish. Res. Board Can. 29: 588–589.

MARSH, M. C., AND F. P. GORHAM. 1905. The gas disease in fishes. Rep. Bur. Fish. 1904: 343–376.

NEBEKER, A. V., AND J. R. BRETT. 1976. Effects of gas supersaturated water on survival of Pacific salmon and steelhead smolts. Trans. Am. Fish. Soc. 105: 2.

NEBEKER, A. V., D. G. STEVENS, AND J. R. BRETT. 1975. Effects of gas supersaturated water on fresh water aquatic invertebrates. Proc. of Gas-Bubble Disease Workshop, Oct. 8–9, 1974, Battelle-NW, Richland, Wash. p. 1–63.

PAULEY, G. B., AND R. E. NAKATANI. 1967. Histopathology of "gas bubble" disease in salmon fingerlings. J. Fish. Res. Board Can. 24: 867–871.

RUCKER, R. R. 1972. Gas bubble disease of salmonids: a critical review. U.S. Bur. Sport Fish. Wildl., Tech. Pap. 11 p.

RUCKER, R. R., AND K. HODGEBOOM. 1953. Observations on gas bubble disease of fish. Prog. Fish Cult. 15: 24–26.

SAMUELSON, D. 1976. Seasonal comparison of basic water quality for the Western Fish Toxicology Station, the Willamette River and major Western Oregon Rivers (1972–1974). U.S. Environ. Prot. Agency Res. Ser. (In press)

WEITKAMP, D. E., AND M. KATZ. 1973. Resource and literature review. Dissolved gas supersaturation and gas bubble disease. Seattle Marine Laboratory, Seattle, Wash. 60 p.

WESTGARD, R. L. 1964. Physical and biological aspects of gas bubble disease in impounded adult chinook salmon at McNary spawning channel. Trans. Am. Fish. Soc. 93: 306–309.